Polish Journal of Microbiology
2019, Vol. 68, No 1, 93–104
https://doi.org/10.21307/pjm-2019-011

ORIGINAL PAPER

New Insight into Genotypic and Phenotypic Relatedness of *Staphylococcus aureus* Strains from Human Infections or Animal Reservoirs

KLAUDIA LISOWSKA-ŁYSIAK[1#], MAJA KOSECKA-STROJEK[1#], JOANNA BIAŁECKA[2], ANDRZEJ KASPROWICZ[2], KATARZYNA GARBACZ[3], LIDIA PIECHOWICZ[4], VLADIMIR KMET[5], VINCENZO SAVINI[6] AND JACEK MIĘDZOBRODZKI[1*]

[1] Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[2] Centre of Microbiological Research and Autovaccines, Krakow, Poland
[3] Department of Oral Microbiology, Medical Faculty, Medical University of Gdansk, Gdansk, Poland
[4] Department of Medical Microbiology, Medical Faculty, Medical University of Gdansk, Gdansk, Poland
[5] Centre of Biosciences, Institute of Animal Physiology, Slovak Academy of Sciences, Slovakia
[6] Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara (PE), Italy

Submitted 28 September 2018, revised 22 November 2018, accepted 7 December 2018

Abstract

Staphylococcus aureus is a common human and livestock opportunistic pathogen, and there is evidence of animal to human transmission. This paper aimed to recognize properties of the isolates from collections of human and livestock *S. aureus* strains and to estimate compatibility of results based on phenotypic tests, microarrays and the *spa* typing methods. The second goal was to study differences between human and animal isolates in terms of specificity of their hosts and the strain transmission among various hosts. Most strains showed multi-susceptible profiles and produced enzymes on a high level, and they were phenotypically and genetically similar. However, in contrast to the Polish bovine mastitis strains, the Slovakian strains were multi-resistant. In this research, the strains showed significant differences in terms of their phenotypic manifestations and the presence of hemolysins genes; however, other enzyme-encoding genes correlated to a higher extent with the microarrays results. Interestingly, there was a lack of enterotoxin genes in human Poultry-like protein A+ strains in comparison to other human strains. Our study showed that differences between virulence profiles of the human and animal strains correlated with their origin rather than their hosts, and any trait allowed clearly distinguishing between them based on the microarray results.

Key words: genetic profile, infection, microarrays, phenotype, *Staphylococcus aureus*

Introduction

A common human and animal opportunistic pathogen *Staphylococcus aureus* occurs mostly in the skin and/or nose vestibule mucous membrane as consistent of natural microflora in healthy individuals. Colonization can lead to an invasive mode of infection under particular conditions of a host organism, mostly immunodeficiency, surgical interventions or longitudinal hospitalization of patients (Malachowa et al. 2011; O'Gara 2017). The European Centre for Disease Prevention and Control alarms that the most antimicrobial-resistant healthcare-associated infections are caused by the methicillin-resistant *S. aureus* (MRSA). Estimating the level of distribution, the MRSA isolates are responsible for 10% to 25% of all staphylococcal infections in Poland; in Slovakia, these rates are even higher (25% to 50%). Moreover, an increasing problem is the expansion of community-acquired MRSA (ECDC 2015).

Staphylococcal strains carrying antimicrobial-resistance genes colonize a vast range of animal species, especially household or livestock animals, with clear evidence of the intra-species transmission of staphylococci (Angen et al. 2017; Kmet et al. 2018).

Under particular conditions, *S. aureus* causes infections, and become high-risk pathogens due to several virulence determinants such as toxins and enzymes combined with other survival strategies of bacteria

Equal shares in the paper composition.

* Corresponding author: J. Międzobrodzki, Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland; **e-mail: jacek.miedzobrodzki@uj.edu.pl**

like antibiotic resistance and biofilm production (Goldmann and Medina 2017). An important staphylococcal strategy is to damage host cell membranes, caused by hemolysins, bi-component leukocidins, and phenol soluble modulins. For example, α-hemolysin and bi-component leukocidins act as pore-forming toxins, while β-haemolysin is sphingomyelinase, which hydrolyzes lipids of plasma membrane into ceramide and phosphorylcholine (Herrera et al. 2016). Additionally, α-hemolysin and Panton-Valentine leucocidin (PVL) promote apoptosis of phagocytes (Seilie and Bubeck Wardenburg 2017). Another group of virulence factors is an enterotoxin superfamily. These toxins are responsible for staphylococcal food poisoning, but they also affect some immune system cells, with further consequences (Zhang et al. 2017).

S. aureus strains also secrete a variety of enzymes. Proteases include a vast group of secreted enzymes, such as aureolysin, serine proteases, and staphopains, which are engaged in the evasion of complement-mediated bacterial killing (Miedzobrodzki et al. 2002; Sabat et al. 2008; Otto 2014). Nuclease, another extracellular enzyme, degrades neutrophil extracellular traps (NETs), providing the strain resistant to NET-mediated killing (Zawrotniak and Rapala-Kozik 2013; Paharik and Horswill 2016). *S. aureus* also produces lipase, which lyses triglycerides to free fatty acids – but the biological function of this process is still unknown (Cadieux et al. 2014). Finally, extracellular urease is involved in biofilm regulation and protection against low pH. Urease catalyzes the hydrolysis of urea and neutralizes acids using ammonia (Vandecandelaere et al. 2017). Triggering calculus formation, an increased pH level plays a significant role in urinary tract diseases (Paharik and Horswill 2016).

The progress of *S. aureus* infections depends on the secretion of surface proteins, numerous extracellular toxins, and enzymes that destruct host cells and tissues (Kong et al. 2016). Due to this fact, this work aimed to recognize the characteristic properties of *S. aureus* strains based on phenotypic and genetic features such as the production of enzymes and toxins, their resistance profiles and MRSA detection. Firstly, the strains were genetically characterized by microarrays' profiling and *spa* typing. Secondly, the human and animal strains were compared based on microarray testing and phenotypic manifestations, together with the analysis of their relatedness or differences between them.

Experimental

Materials and Methods

Strains collection. Fifty-three not duplicate *S. aureus* strains were isolated from deep tissue infections of ambulatory patients and from animals (Table I). The number of strains was sufficient to group the strains based on their similarities, to define dominant properties of the strains and groups, and to compare them. The 26 human strains were obtained from four medical institutions and universities: (i) 11 isolates from the Centre of Microbiological Research and Autovaccines and different hospital wards in Kraków (Poland), (ii) 11 Poultry-like protein A positive (P-like pA+) isolates from the Medical University of Gdańsk, (Poland), and (iii) four isolates from the Spirito Santo Hospital, Pescara (Italy). Among these strains, 21 isolates originated from the following infections: wound infections (n = 9), boils (n = 3), deep skin lesions (n = 2), ulcers (n = 2), conjunctivitis (n = 2), blood (n = 1), cyst (n = 1), and pus (n = 1); the other five were isolated from throats (n = 3) and nose swabs (n = 2). Moreover, the collection was enriched with 11 P-like pA+ strains isolated from patients in Gdańsk, an atypical origin of P-like pA+ biotype, although there is no information about the link between these strains occurrence and patient's employment. Interestingly, these strains are usually detected in meat products, in people having direct contact with meat, and in the places where fresh meat occurs in abundance (Piechowicz and Garbacz 2016).

The 27 animal strains were isolated from animals, which had daily physical contact with people. The strains were obtained from four veterinary institutions and universities: (i) 15 bovine isolates from the Faculty of Biology and Animal Breeding, University of Life Sciences, Lublin (Poland); (ii) seven bovine isolates from the Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice (Slovakia); (iii) four canine isolates from the Medical University of Gdańsk (Poland); and (iv) one canine isolate from the Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wrocław (Poland). Only one animal strain was isolated from throat infection of a dog while the others were isolated from eczema, eye, or skin infections. All the strains were isolated from animals inhabiting human environments with frequent contact with people what facilitates the inter-genus transmission of bacteria.

Taking into consideration a widespread and active transmission of staphylococci, the bacteria from various sources and different geographic regions were used in this research.

Antibiotic susceptibility testing. Before molecular analysis the antibiotic susceptibility testing was performed using the disc diffusion according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2017). The bacteria were cultivated for 24 hours at 37°C on MH agar plates with antibiotic discs. The 24 h incubation is required for cefoxitin susceptibility testing according to according to EUCAST (Żabicka and Hryniewicz 2009). The strains were tested using a

Table I
A list of the strains collected, their origin and place of isolation.

Strain no.	Host	Lesions/Material	Place of origin
1	human	wound infection	Kraków (Poland)
2	human	cyst	Kraków (Poland)
3	human	wound infection	Kraków (Poland)
4	human	wound infection	Kraków (Poland)
5	human	wound infection	Kraków (Poland)
6	human	wound infection	Gdańsk (Poland)
7	human	wound infection	Gdańsk (Poland)
8	human	boil	Gdańsk (Poland)
9	human	boil	Gdańsk (Poland)
10	human	boil	Gdańsk (Poland)
11	human	throat	Gdańsk (Poland)
12	human	pus	Gdańsk (Poland)
13	human	throat	Gdańsk (Poland)
14	human	throat	Pescara (Italy)
15	human	skin infection	Pescara (Italy)
16	human	blood	Pescara (Italy)
17	human	skin infection	Pescara (Italy)
18	human	nose swab	Gdańsk (Poland)
19	human	ulcer	Gdańsk (Poland)
20	human	ulcer	Gdańsk (Poland)
21	human	wound infection	Kraków (Poland)
22	human	wound infection	Kraków (Poland)
23	human	conjunctivitis	Kraków (Poland)
24	human	conjunctivitis	Kraków (Poland)
25	human	wound infection	Kraków (Poland)
26	human	nose swab	Kraków (Poland)
27	animal	eczema	Gdańsk (Poland)
28	animal	eye	Gdańsk (Poland)
29	animal	eye	Gdańsk (Poland)
30	animal	throat	Gdańsk (Poland)
31	animal	skin infection	Wrocław (Poland)
32	animal	mastitis	Košice (Slovakia)
33	animal	mastitis	Košice (Slovakia)
34	animal	mastitis	Košice (Slovakia)
35	animal	mastitis	Košice (Slovakia)
36	animal	mastitis	Košice (Slovakia)
37	animal	mastitis	Košice (Slovakia)
38	animal	mastitis	Košice (Slovakia)
39	animal	mastitis	Łęczna (Poland)
40	animal	mastitis	Łęczna (Poland)
41	animal	mastitis	Lubartów (Poland)
42	animal	mastitis	Łęczna (Poland)
43	animal	mastitis	Łęczna (Poland)
44	animal	mastitis	Łuków (Poland)
45	animal	mastitis	Łuków (Poland)
46	animal	mastitis	Gawrolin (Poland)
47	animal	mastitis	Świdnik (Poland)
48	animal	mastitis	Świdnik (Poland)
49	animal	mastitis	Świdnik (Poland)
50	animal	mastitis	Tomaszów Lubelski (Poland)
51	animal	mastitis	Tomaszów Lubelski (Poland)
52	animal	mastitis	Tomaszów Lubelski (Poland)
53	animal	mastitis	Tomaszów Lubelski (Poland)

set of 12 antibacterial agents, including the following: i) aminoglycosides: amikacin and gentamicin; ii) beta-lactams: amoxicillin with clavulanic acid, cefoxitin, and penicillin; iii) fluoroquinolones: ciprofloxacin and norfloxacin; iv) lincosamides: trimethoprim/sulfamethoxazole; v) macrolides: clindamycin and erythromycin; and others: chloramphenicol and doxycycline. The diameter of the transparent zones of growth inhibition was measured, and clinical breakpoints were evaluated.

Evaluation of enzymatic activity. A single colony of each bacterial strain was transferred from a TSA agar plate (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) to specific media, including substrates dedicated to particular enzymes: a TSA agar plate supplemented with 10% of skim milk proteins; a blood agar plate with 5% sheep blood (Graso Biotech, Starogard Gdański, Poland); a TSA agar plate enriched with 2% Tween 80; 10% of 1M $CaCl_2$; DNase test agar (Becton Dickinson, New Jersey, USA); and Christensen's Urea Agar Base (REFE112L), containing 40% of Urea Solution (EBO48), (Becton Dickinson, New Jersey, USA) for proteolysis testing, hemolysis testing, lipases activity testing, nucleases activity testing, and ureases activity testing, respectively. Bacterial strains on appropriate plates were then cultured for 24 hours at 37°C. To evaluate proteolysis activity, the diameters of transparent zones around the colonies were measured (Puacz et al. 2015). To rate types of hemolysis the transparent zones for β-hemolysis (including the second zone observed in double β-hemolysis) and dark-green opalization for α-hemolysis were evaluated (Puacz et al. 2015). Lipase activity was measured by the size of a turbidity zone around colonies, nuclease activity – by the size of a transparent zone after HCl addition to the culture test, while urease activity – by the color change of the medium from yellow to purple (Black et al. 1971; dos Santos Rodrigues et al. 2014; Posteraro et al. 2015). To evaluate enzymatic activity, a sample was assessed

as either negative (no activity) or positive (with low, moderate, high or very high activity).

Molecular techniques. The *spa* typing. The *spa* typing technique was used following Aires-de-Souza et al. (2006). The method is based on the sequence analysis of amplified fragments of the X region of the protein A gene, resulting in *spa* types, assigned by the Ridom StaphType software version 2.1.1 (Ridom GmbH, Würzburg, Germany) and the Ridom SpaServer (http://www.spaserver.ridom.de). Based on *spa* types, *spa* clonal complexes (*spa*-CCs) are then calculated using the Based Upon Repeat Pattern (BURP) algorithm with the following parameters: (i) no exclusion criteria related to the number of repeats were used; (ii) cost equal to 4; (iii) a cluster composed of 2 or more related *spa* types was regarded as a clonal complex (CC); and (iv) a *spa* type that was not grouped into a CC was considered a singleton.

Microarray analysis. Microarray assays were done using the StaphyType system (StaphyType, Alere Technologies, Jena, Germany). The StaphyType kit allows the simultaneous detection of 334 *S. aureus* target sequences, including approximately 170 distinct genes and their allelic variants. The DNA microarray procedures were carried out according to the manufacturer's instructions. According to Monecke's et al. (2008) methodology, the strains were automatically assigned to CCs or sequence types (STs) by the StaphyType software. The results for individual genes were converted into the following scale: A = positive, T = negative, and C = ambiguous.

Results

Antibiotic susceptibility testing. All the *S. aureus* strains tested were susceptible to trimethoprim/sulfamethoxazole (Table II). Five human (19%) and ten animal (37%) strains were susceptible to all the antibiotics tested. Eight strains (15%), the Slovakian bovine strains together with one human strain originated from Kraków were multi-resistant (resistant to more than three antibiotics). The highest resistance rate was observed for penicillin (n = 33; 62%), with 17 human (65%) and 16 animal (59%) resistant strains. The detection of resistance to cefoxitin helped to identify nine strains (two from humans and seven from animals) as methicillin-resistant (MRSA), and one of them did not exhibit resistance to benzylpenicillin/ amoxicillin. Among the MRSA strains, bovine strains from Slovakia (n = 7) were resistant to other antibiotics. All of these strains demonstrated resistance to erythromycin and clindamycin, and they were classified to have an inducible MLS_B (macrolides-lincosamides-streptogramin B resistance) phenotype. Bovine strains of non-Slovakian origins were susceptible to the majority of antibiotics tested. Among methicillin-susceptible *S. aureus* (MSSA) strains, 18 (54%) demonstrated resistance only to penicillin/amoxicillin, two (6%) to penicillin and doxycycline, four (12%) to penicillin and erythromycin, and one to chloramphenicol and aminoglycosides. The susceptibility rate was high for both human and animal isolates. In both collections, however, the proportions of strains resistant to antimicrobials other than β-lactams were equal.

Table II
Antibiotic susceptibility of *S. aureus* strains. MRSA strains are bolded in text.

Antibiotic	Number of strains		
	Human (%) n = 26	Animal (%) n = 27	All (%) n = 53
Resistant to all antibiotics tested	0 (0)	0 (0)	0 (0)
Multi-resistant	1 (4)	7 (26)	8 (15)
BEN	17 (65)	16 (59)	33 (62)
AMC	2 (8)	7 (26)	9 (17)
FOX	**2 (8)**	**7 (26)**	**9 (17)**
SXT	0 (0)	0 (0)	0 (0)
CHL	1 (4)	1 (4)	2 (4)
DOX	3 (12)	1 (4)	4 (8)
ERY	6 (23)	8 (30)	14 (26)
CLI	0 (0)	7 (26)	7 (13)
AMI	2 (8)	2 (7)	4 (8)
GEN	2 (8)	0 (0)	2 (4)
CIP	0 (0)	4 (15)	4 (8)
NOR	0 (0)	4 (15)	4 (8)
Susceptible to all antibiotics tested	5 (19)	10 (37)	15 (28)

BEN: benzylpenicillin, AMC: amoxicillin with clavulanic acid, FOX: cefoxitin, SXT: trimethoprim/sulfamethoxazole, CHL: chloramphenicol, DOX: doxycycline, ERY: erythromycin, CLI: clindamycin, AMI: amikacin, GEN: gentamicin, CIP: ciprofloxacin, NOR: norfloxacin. Multi-resistant – resistant to more than three antibiotics.

Enzymatic activity. In the *S. aureus* collection, 42 strains (79%) demonstrated β-hemolytic activity: 18 strains showed a double zone of hemolysis (Savini et al. 2013), and 24 strains presented a single zone of hemolysis; 11 strains (21%) did not demonstrate hemolysis (Fig. 1.1). Double-zone hemolysis occurred mostly in human strains (n = 12, 23%), and 17 animal strains showed regular β-hemolysis zone. Both human and animal populations showed similar number of β-hemolytic strains.

Fifty-two (98%) strains demonstrated proteolytic activity. Most of the strains showed low (n = 21, 40%) or moderate (n = 23, 43%) proteolysis. Seven (13%) strains, of which five were the human origin, showed high proteolytic activity. Most human (n = 12) and ani-

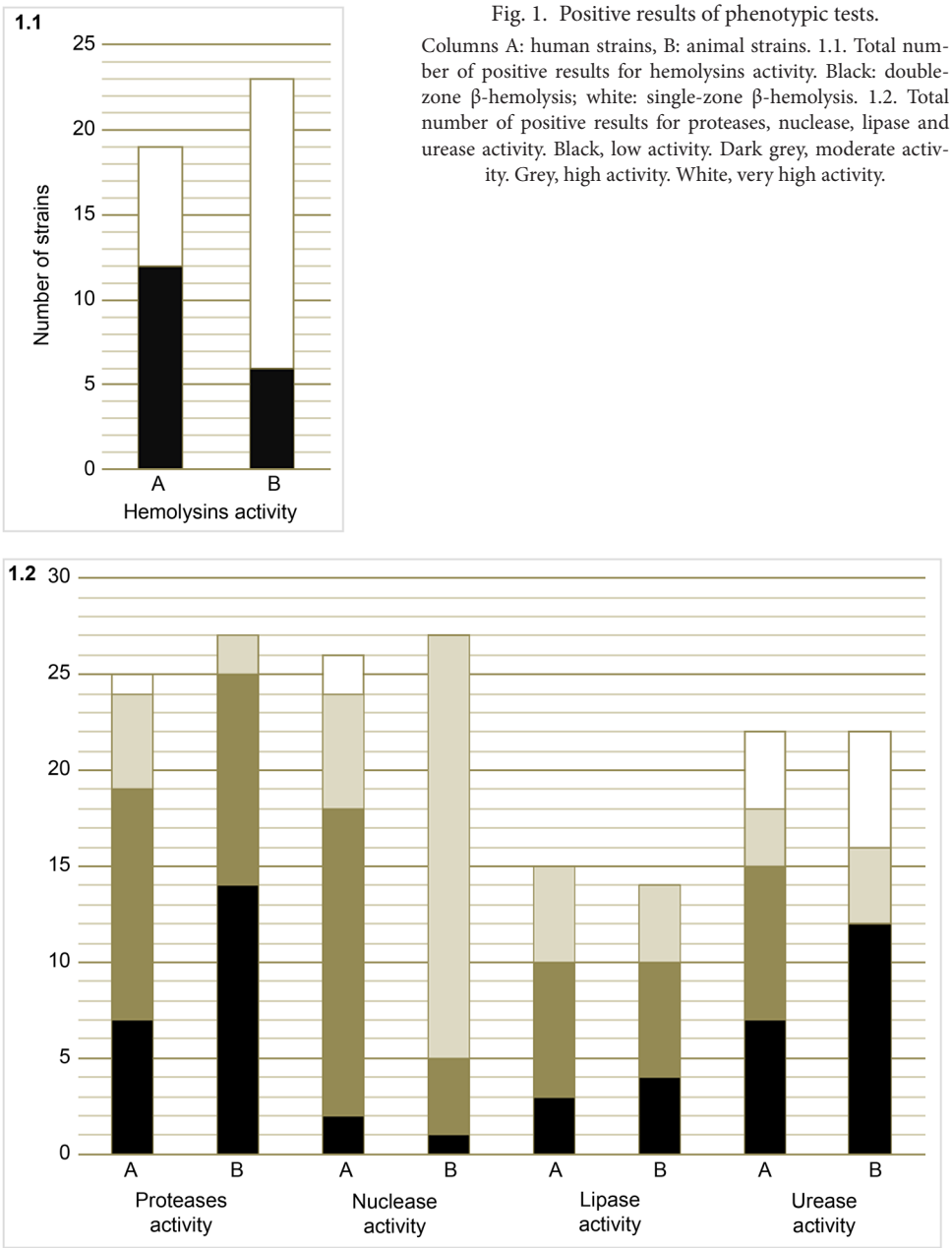

Fig. 1. Positive results of phenotypic tests.
Columns A: human strains, B: animal strains. 1.1. Total number of positive results for hemolysins activity. Black: double-zone β-hemolysis; white: single-zone β-hemolysis. 1.2. Total number of positive results for proteases, nuclease, lipase and urease activity. Black, low activity. Dark grey, moderate activity. Grey, high activity. White, very high activity.

mal (n = 11) strains showed moderate while 14 animal strains (n = 14) showed low proteolytic activity (Fig. 1.2).

All the strains showed nuclease activity, usually high (n = 28, 53%) or moderate (n = 20, 38%). The animal strains (n = 22; 81%) showed higher nuclease activity than human strains, which mostly exhibited moderate activity (n = 16; 62%).

Twenty-nine (55%) strains showed lipase activity, high activity was presented by nine strains (16%; five were human and four animal), moderate activity exhibited 13 strains (25%; seven were human and six animal), and low activity – seven strains (13%; three were human and four animal). Twenty-four (45%) strains did not exhibit any lipase activity. The human and animal bacteria collections showed an almost identical rate of lipase activity.

Forty-four (83%) strains demonstrated urease activity, but in most cases (n = 19, 36%) it was low. Six animal and four human strains showed very high urease activity (n = 10, 19%), three human and seven animal strains showed high activity (n = 10, 19%), and eight human strains showed moderate activity (15%). Nine strains (five from animals and four from humans; n = 9; 17%) did not exhibit urease activity. Human and animal strains did not differ in terms of urease activity. Figure 1 shows the results of phenotypic testing.

The *spa* typing. Based on the *spa* typing, 35 *S. aureus* strains were assigned into 20 *spa* types (Table III). Eighteen singletons were found, including the following *spa* types: t053, t091, t127, t150, t335, t723, t793, t3165, t4087, t5447, t14393, t14394, t14403, and t14404. Table III shows bacterial isolates assigned to *spa* types

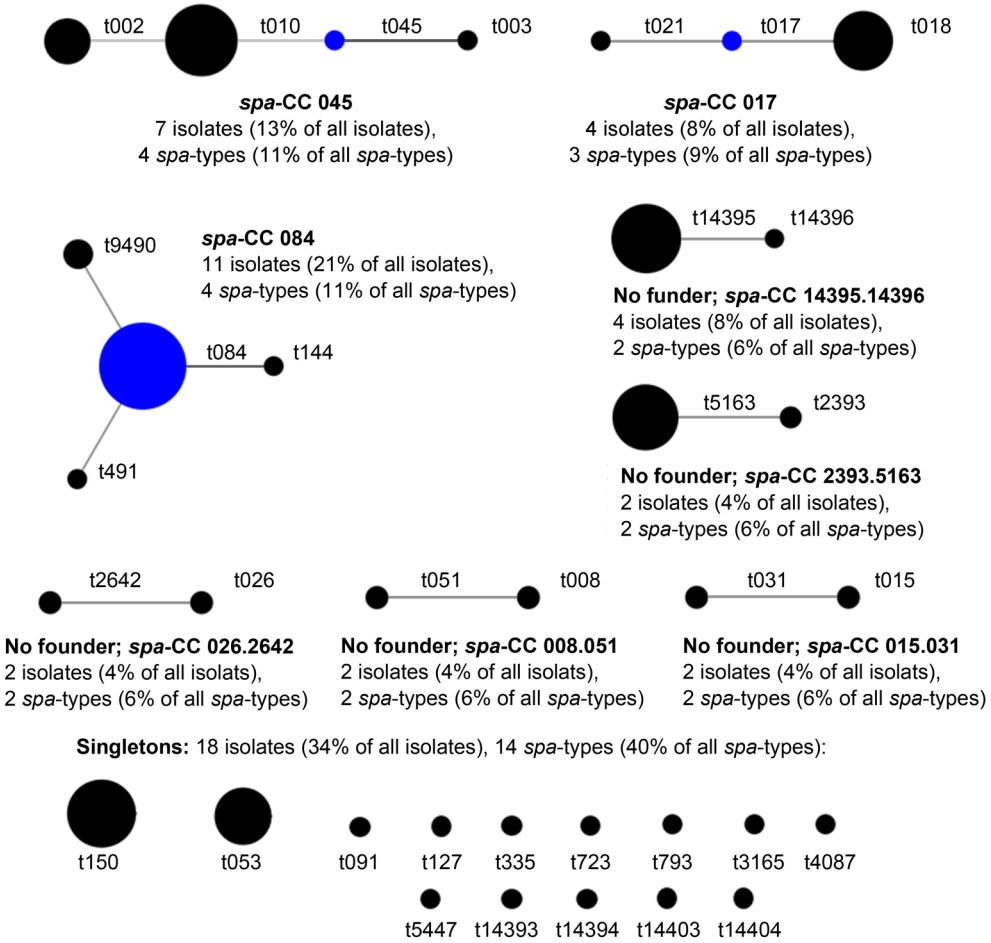

Fig. 2. The results of BURP analysis. The 20 *spa* types were assigned to proper clonal complexes. Eighteen singletons were found among the 53 strains analyzed. Black circles represent the *spa* types assigned to the proper clonal complexes, and the blue circles represent the ancestor within the clonal complex.

and clonal complexes together with their resistance and virulence genes. Figure 2 presents the results of BURP analysis, which demonstrates the clonal complexes based on the *spa* gene sequence similarities. Interestingly, *spa*-CC 045 was previously described as *spa*-CC 002 using Ridom StaphType software version 1.4 (Ridom GmbH, Würzburg, Germany), but a further update to version 2.1.1 resulted in changing the founder strain within the clonal complex.

The human strains belonged into more different *spa*-CCs than animal strains did. Most human strains (n = 10) and only one animal strain were assigned to *spa*-CC 084. Interestingly, these ten human strains and one canine strain originated from the same city Gdańsk. Other *spa*-CCs of human strains were as follows: *spa*-CC 008.051 (n = 2), *spa*-CC 017 (n = 3), *spa*-CC 015.031 (n = 2), and *spa*-CC 026.2642 (n = 2). Among the animal strains, *spa*-CCs were also distinct, but there was a strong correlation between *spa*-CC and a place of strain isolation. The most numerous clonal complex was *spa*-CC 084 (n = 11). The geographic *spa* type division was distinctly observed among the strains originating from bovine mastitis infections. Four bovine strains originating from Tomaszów Lubelski were assigned to one *spa*-CC 14395.14396, four strains originating from Łęczna to *spa*-type t150, and two strains originating from Łuków to *spa*-type t053. Among the bovine strains received from Košice (Slovakia), two *spa*-CCs were reported: *spa*-CC 045 (n = 4) and *spa*-CC 2393.5163 (n = 3). The former *spa*-CC was also identified in Świdnik (n = 3).

Microarray testing. All the strains demonstrated positive results for the species markers: *gapA* (glyceraldehyde 3-phosphate dehydrogenase), *katA* (catalase), *coa* (coagulase), *spa* (*Staphylococcus* protein A), *sbi* (IgG-binding protein), *nuc* (thermostable nuclease), *fnbA* (fibronectin-binding protein A), *vraS* (sensor protein), *sarA* (staphylococcal accessory regulator A), *eno* (enolase), and *saeS* (histidine protein kinase). The presence of *nuc* gene was also confirmed phenotypically (Fig. 1). Five strains presented negative results, and two strains – ambiguous results for the 23S-rRNA gene, and they were probably caused by RNA contamination. The results for these strains were reliable because the

Table III
The results of the *spa* typing. Bacterial isolates were assigned to *spa* types and clonal complexes (CCs).

Strain number	*spa* types	Sequence type (ST)*	Clonal complex	Resistance and virulence genes
26	t008	ST-8, ST-427, ST-250, ST-254	*spa*-CC 008.051	Human strains: 26/23 Resistance: ***blaZ/I/R*** **(+/–)**, *tetEfflux, fosB* Enterotoxins: ***sea*** **(–/+),** ***sed*** **(+/–),** ***seg*** **(+/–),** ***sej*** **(+/–),** ***ser*** **(+/–)** Enzymes within hemolysins: *sak, hla, hlb,* ***lukM/luk F*** **(+/–)**, *hlgA, lukD, lukE, lukX,* Proteases: *aur, splA, splB, splE* Adhesion and biofilm formation: *bbp, fnbB, map, sdrC, sdrD, sas*G
23	t051	ST-250, ST-254		
4	t015	ST-45	*spa*-CC 015.031	Human strains: 4/21 Resistance: ***mecA*** **(+/–)**, *blaZ/I/R,* ***erm(A)*** **(–/+)**, *tetEfflux* Enterotoxins: ***seb*** **(–/+),** *sec, seg,* ***sej*** **(+/–)**, ***sel*** **(–/+)**, *sei, sem, sen, seo, seu, egc-cluster* Enzymes within hemolysins: *sak, hla, hlgA, lukX,* Proteases: *aur* Adhesion and biofilm formation: *bbp, cna, fnbB,* ***map*** **(+/–)**, sdrC, sdrD
21	t031	ST-45		
25	t017	nd	*spa*-CC 017	Human strains: 25/5/ animal strain: 31 Resistance: ***blaZ/I/R*** **(–/+/+)**, ***erm(A)*** **(–/+/–)**, ***tet(K)*** **(–/–/+)**, *tetEfflux, fosB* Enterotoxins: *seg, sei, sem, sen, seo, seu, egc-cluster* Enzymes within hemolysins: ***sak*** **(+/+/–)**, *hla, hlb, hlgA,* ***lukX*** **(+/–/–)**, ***lukX*** **(–/–/+)** Proteases: *aur, splE* Adhesion and biofilm formation: *bbp, cna,* ***fnbB*** **(+/+/–)**, ***map*** **(–/+/+)**, *sdrC, sdrD*
5	t021	ST-30, ST33, ST-55		
16, 31	t018	ST-30, ST36, ST-38		
15	t026	ST-45, ST-47	*spa*-CC 026.2642	Human strains: 15/2 Resistance: ***mecA*** **(–/+)**, ***erm(C)*** **(–/+)**, ***addD*** **(–/+)**, ***mupR*** **(–/+)**, *tetEfflux,* ***fosB*** **(+/–)**, ***cat*** **(–/+)** Enterotoxins: *sec, seg, sei,* ***sel*** **(+/–)**, *sem, sen, seo, seu, egc-cluster* Enzymes within hemolysins: *sak, hla, hlgA,* ***lukX*** **(–/+)** Proteases: *aur* Adhesion and biofilm formation: ***bbp*** **(–/+)**, ***cna*** **(+/–)**, *fnbB, map, sdrC,* ***sdrD*** **(–/+)**
2	t2642	nd		
50, 51, 52, 53	t14395	nd	*spa*-CC 14395.14396	Animal strains: 50/51/52/53 Resistance: *tetEfflux, fosB* Enterotoxins: *seg, sei, sem, sen, seo, seu, egc-cluster* Enzymes within hemolysins: *hla, hlb, hlgA, lukX* Proteases: *aur* Adhesion and biofilm formation: *bbp, fnbB, map, sdrC,* ***sdrD*** **(51, 53: +, 50, 52: –)**
32, 34	t002	ST-5, ST-231	*spa*-CC 045	Animal strains: 32/34/33/35 Resistance: ***mecA, blaZ/I/R*** **(strain no. 34: –)**, ***erm(A)*** **(strains no. 34, 35: +),** *addD, tetEfflux,* ***cat*** **(strain no. 35: +)**, *fosB* Enterotoxins: ***sea*** **(strains no. 33, 35: +)**, ***sed*** **(strain no. 34: –)**, *seg, sei,* ***sej*** **(strain no. 34: –)**, *sem, sen, seo,* ***ser*** **(strain no. 34: –)**, *seu, egc-cluster* Enzymes within hemolysins: *sak, hla, hlb, hlgA, lukD, lukE, lukX* Proteases: *aur, splA, splB* Adhesion and biofilm formation: *bbp,* ***fnbB*** **(strains no. 33, 35: +),** *map, sdrC, sdrD, sasG*
33	t003	ST-5, ST-225		
35	t045	ST-5, ST-225		
47, 48, 49	t010	ST-5		Animal strains: 47/48/49 Resistance: *blaZ/I/R, tetEfflux, fosB* Enterotoxins: *seg, sei, sem, sen, seo, seu, egc-cluster* Enzymes within hemolysins: *hla, hlb, hlgA, lukD, lukE, lukX* Proteases: *aur*, *splA*, *splB* Adhesion and biofilm formation: *bbp, map, sdrC, sdrD, sasG*

Table III. Continued

Strain number	*spa* types	Sequence type (ST)*	Clonal complex	Resistance and virulence genes
1, 7, 10, 11, 12, 18, 19	t084	ST-15, ST-18	*spa*-CC084	Human strains: 1/7/10/11/12/18/19/8/20 Animal strain: 28 Resistance: ***blaZ/I/R*** **(strains no. 12, 18: –)**, ***tet(K)*** **(strains no. 7, 12, 19, 20: +)**, *tetEfflux*, ***fosB*** **(strain no. 7: –)** Enterotoxins: – Enzymes within hemolysins: ***sak*** **(strain no. 28: +)**, ***hla*** **(strains no. 10, 28: –)**, ***lukM/lukF*** **(strains no. 19, 20: +)**, ***hlgA*** **(strains no. 10, 28: –)**, ***lukD*** **(strain no. 10: –)**, ***lukE*** **(strains no. 18, 19: +)**, ***lukF*** **(strain no. 20: +)**, ***lukX*** **(strains no. 18, 19, 20: +)** Proteases: ***aur*** **(strains no. 10, 11, 28: –)**, *splA, splB,* ***splE*** **(strains no. 10, 28: –)** Adhesion and biofilm formation: *bbp* **(strains no. 10, 8: –)**, *fnbB* **(strain no. 11: –)**, *map, sdrC, sdrD, sasG*
8,9	t9490	nd		
28	t144	nd		
20	t491	nd		
38	t2393	nd	*spa*-CC 2393.5163	Animal strains: 38/36/37 Resistance: ***mecA***, *blaZ/I/R, erm(C), tet(K), tetEfflux* Enterotoxins: *sea* Enzymes within Hemolysins: *sak, hla, hlb, hlgA, lukD, lukE, lukX* Proteases: *aur, splA, splB* Adhesion and biofilm formation: *bbp, fnbB, map, sdrC, sdrD, sasG*
36, 37	t5163	nd		

* The proposed sequence type retrieved from Ridom SpaServer. Genes exhibited by all the analyzed strains: hemolysins: *hld*, *luk F*, *lukS*, *lukY*; proteases: *sspA*, *sspB*, *sspP*; adhesion and biofilm formation: *icaA*, *icaC*, *icaD*, *clfA*, *clfB*, *ebh*, *eno*, *fib*, *ebpS*, *fnbA*, *vwb.*

number of ambiguous results did not exceed 3%. All isolates but one (n = 52; 98%) carried the *tetEfflux* gene, which encodes tetracycline efflux protein, and the *fosB* gene (n = 43; 81%), which confers resistance to fosfomycin and bleomycin. In 32 strains (17 from humans and 15 from animals), the genes involved in penicillin resistance by the production of β-lactamase (*blaZ*, *blaI*, and *blaR*) were detected; among these strains, eight were MRSA strains. Strains carrying the other resistance genes did not exceed 17%. The most prevalent resistance patterns were: *tetEfflux, fosB, and blaZ/I/R, tetEfflux, fosB* (Table III). The first one was carried by strains belonged to *spa*-CC 084 (n = 6; t084, t144, t9490), *spa*-CC 045 (n = 3; t010), and *spa*-CC 008.015 (n = 1, t008). Strains with *spa* type t010 were the only MSSA strains from the *spa*-CC 045. The other strain from *spa*-CC 008.015 (no. 23; t051), and two strains from *spa*-CC 084 (no. 9, 18), harbored the second most frequent pattern. The same resistance genes were present also in *spa*-CC 017 (n = 1; t021), and all of the strains from *spa*-CC 14395.14396. The animal strains of *spa*-CC 2393.5163 carried a unique resistance pattern among other strains from the collections (Table III).

These MRSA strains carried *mecA* or *mecR*, and associated with the SCC*mec* element genes for glicerophosphoryl-diester-phosphodiesterase (*upgQ*), and cassette chromosome recombinases (*ccrA-2*, *ccrB-2*). Four of these strains carried also potassium-transporting ATPases (*kdp* operon); a DNA-binding response regulator; the *mecI* gene; and *xylR*, encoding a pseudogene of xylose repressor. The most MRSA strains belonged to *spa*-CC 045 (n = 4), and *spa*-CC 2393.5163 (n = 3). Two strains carried the *mecA* gene and they were *blaZ/I/R* negative. One of them exhibited *spa* type t002 (*spa*-CC 045), and the other belonged to *spa* type t2642 (*spa*-CC 026.2642) (Table III).

All the 53 isolates harbored the δ-hemolysin gene, components of other hemolysins, and the leucocidin D component (*lukF/S*, *lukY*, *hl*, *hld*, *hldIII*). All but three strains carried also the gene for α-hemolysin (*hlgA*, n = 50; 94%); the β-hemolysin gene (*hlb*, n = 35, 66%); and the staphylokinase gene (*sak*, n = 24; 45%). The absence of both – *hlb* and *sak* genes was a characteristic feature of P-like pA+ genotype of the strains in comparison to other human strains which carried *hlb* (n = 11) and *sak* (n = 12) gene in more than 70%. Lack of the *sak* genes were observed also in animal stains from t010, and t14395. On the other hand, the *hlb* gene was absent in human *spa*-CCs 026.2642 and 015.031. Human P-like pA+ strains were in majority grouped in *spa*-CC 084 (t084, t9490, t491), and two of them were classified as singletons (t335, t793). The characteristic property of these stains was also lack of enterotoxins genes.

Fifteen enterotoxin genes frequently occurred in human and animal strains from most regions, including *sea* (n = 11; 21%), *seb* (n = 2; 4%), *sec* (n = 3; 6%) *sed* (n = 5; 9%), *seg* (n = 25; 47%), *seh* (n = 1; 2%), *sei* (n = 24; 45%), *sej* (n = 5; 9%), *sel* (n = 3; 6%), *selm* (n = 24; 45%),

seln (n = 24; 45%), *selo* (n = 24; 45%), *ser* (n = 5; 9%), *selu* (n = 24; 45%), and *ego*-cluster (n = 24, 45%), which encodes enterotoxins *seg*, *sei*, *selm*, *seln*, *selo*, and *selu.* Although, strains from *spa* types t008, t2393, and t5163 carried only enterotoxin A gene (*sea*).

All the 53 isolates harbored the V8-protease gene (*sspA*) and staphopain A and B (*sspP*, *sspB*), and 47 strains (89%) carried the aureolysin gene (*aur*). Genes for serine proteases also were detected frequently: 20 human and 21 animal strains (n = 41; 77%) carried the *splA* and *splB* genes. Additionally, 10 animal and 20 human strains (n = 30; 57%) carried the *splE* gene, encoding serine protease E.

Adhesin and biofilm formation genes frequently occurred in the strains analyzed. All of them exhibited following genes: *icaA*/*C* (intercellular adhesion protein A/C), *icaD* (biofilm PIA synthesis protein D), *clfA*/*B* (clumping factor A/B), *ebh*/*ebpS* (cell wall-associated fibronectin-binding proteins), *eno* (enolase), *fib*/*fnbA* (fibrinogen binding proteins), and *vwb* (Willebrand factor). Strains from *spa*-CC 015.031, and *spa*-CC 017 carried *cna* (collagen-binding adhesin) genes, which differentiated them from other strains from the collections.

All the 53 isolates harbored the clumping factor genes (*clfA*, *clfB*), cell wall-associated fibronectin-binding protein gene (*ebh, ebpS*), enolase gene (*eno*), fibrinogen-binding protein (*fib*), fibronectin-binding protein (*fnb*), immunodominant antigen B (*isaB*), heme/transferrin-binding protein (*isdA*), putative transporter protein (*ImrP*), and hyaluronate lyase A1 (*hysA1*). The capsule-5 encoding gene (*capsule 5*) occurred mostly in animal strains while the capsule-8 encoding gene's (*capsule 8*) rate was higher in human strains.

Discussion

The research presented here aimed to characterize the properties of isolates of two particular collections of human and livestock *S. aureus* strains, using phenotypic and genetic methods, and to assess the compatibility of results between microarray and phenotypic manifestations. The investigation focused on the comparison of human and animal strains because studies have shown that animals are a reservoir of pathogens for people (Petinaki and Spiliopoulou 2012). Therefore, dogs have been reported as hosts for MRSA strains, genetically closely related to human strains (Strommenger et al. 2006; Nienhoff et al. 2009).

The next group of animal strains originated from cows. Transmission of bovine strains from cows and cow milk to people and the other way round has been reported as a result of close contact in the dairy environment (Schmidt et al. 2017). Other authors reported a high share of nasal MRSA colonization among volunteers working in farms. However, only 6% MRSA contaminations survive more than 48 hours (Angen et al. 2017). It has been shown that high persistence of multi-resistant isolates increases the importance of monitoring of intra-species strains transmission (Petinaki and Spiliopoulou 2012).

Another way of bovine strains transmission is the contamination of dairy products (Kummel et al. 2016; Nunes and Caldas 2017), and as a result, foodborne infections in human. There was also shown that laboratory investigation detected *S. aureus* in stool samples of 15 patients who had gastrointestinal symptoms, and the strains isolated harbored the *seg*, *sei*, *selm*, *seln*, *selo*, and *selu* genes (Umeda et al. 2017). These results provided the convincing evidence of potential foodborne outbreaks caused by *S. aureus* strains, underlining the significance of bovine strain monitoring to human health prevention.

It was epidemiologically crucial to estimate the resistance to antibiotics for both human and animal pathogens, although the collected strains exhibited low rates of resistance. The low number of MRSA likely resulted from the origin of strains, because all the human strains were received from outdoor patients, and from canine or bovine strains usually presented methicillin-susceptible profiles (Kronenberg et al. 2011; Jagielski et al. 2014). The human and animal collections did not differ in resistance profiles, despite methicillin resistance, which was higher in animal strains.

Detection of the appropriate genes with microarrays also tested all the strains' antibiotic resistance. MRSA strains (n = 9, 17%) were correctly identified by phenotypic antibiotic tests because all of these strains showed the presence of the *mecA* gene. Other genetic traits of antibiotic resistance differed from these phenotypically observed. For the *blaZ*/*I*/*R* genes, such differences occurred for three strains (two from humans and one from an animal). Two of them did not carry *blaZ*/*I*/*R* genes, but phenotyping showed their resistance to penicillin, and one of them presented an opposite correlation (there was no phenotypic resistance and the appropriate genes were present). The second situation probably resulted from the phenotypic method drawback, so the presence of *blaZ*/*I*/*R* genes confirmed by microarrays was recognized as a correct result. For some strains the antibiotic resistance genes presence did not correlate with the phenotypic manifestation: for chloramphenicol (n = 2), doxycycline (n = 6), erythromycin (n = 7), amikacin (n = 3), and gentamicin (n = 7). These data partially agree with results by others in which the occurrence rate of resistance genes was higher than the corresponding phenotypic manifestation of resistance (Li et al. 2015). In the present research, the resistance to antibiotics detected by phenotypic methods only, without any confirmation of the presence of resistance

genes, was possibly a result of mutations or a novel or not tested resistance gene presence.

The next approach was to compare the phenotypic profiles to genetic ones, and for 23 isolates they differed. The *hla* gene was detected in 51 (96%) and *hlb* gene in 35 (66%) strains. These results suggest that hemolysin genes are widespread in staphylococcal populations, even if they do not show any phenotypic manifestation. Thus, there is a strong need to introduce molecular analyses into the hospital and veterinary laboratories, without which many strains may go unnoticed during standard laboratory examinations of patients and ill animals (Moraveji et al. 2014). However, nuclease and protease phenotypic testing gave similar results to molecular analysis.

Furthermore, the human and animal strains were compared based on the microarray patterns, and as it was shown in other investigation, this was the most discriminatory method for strain characterization (Kosecka-Strojek et al. 2016). No specific feature clearly differentiated them when based on the microarray results that concerned resistance genes, virulence genes, adhesion and biofilm formation genes, and immune evasion assay. However, bovine mastitis strains from Poland harbored less resistance and virulence genes than human strains did; this result was in agreement with the recent studies by Schmidt et al. (2017). Interestingly, the bovine strains from Slovakia showed multiresistant profile. Microarray patterns also demonstrated the close similarity between strains originated from the same geographical places of isolation. However, the human P-like pA+ strains originated from Gdańsk showed slightly different microarray patterns than other strains. The reports have shown that the characteristic property of the P-like pA+ strains was lack of *hlb* and *sak* genes, and the present study confirmed this thesis (Piechowicz and Garbacz 2016). Therefore, these strains did not contain any enterotoxin genes in opposite to other human strains of the collections among which most the strains (67%) included genes of *egc*-cluster. Other investigation confirmed a high number of enterotoxin genes (n = 90, 56%) of the strains from Kraków (Ilczyszyn et al. 2016). Interestingly, animal strains from Gdańsk showed a low level of those genes (n = 1). This suggests that the P-like pA+ strains are less virulent than other human strains and that virulence is more comparable to animal strains. As widely known, colonization of various hosts by staphylococci requires adaptive changes, which in turn can be reflected by the acquisition of new genetic characteristics. Against the above data, it cannot be excluded that the P-like pA+ isolates described hereby are animal strains that are at a certain stage of the evolutionary process aimed at transforming them into human strains.

The human and animal strains were also compared based on the *spa* typing, and both collections had different *spa* types and *spa*-CCs. The only exception was *spa*-CC 084, containing ten human strains and an animal strain but all ten strains originated from Gdańsk. Among both human and animal strains, *spa*-CC strongly correlated with the place of strain isolation. Only one *spa*-CC 045 was identified in Świdnik (Poland) and Košice (Slovakia), but according to Ridom SpaServer (www.ridom.de), it is one of the most common *spa*-CCs, with a global frequency of 6.03%. Our study showed that similarities between strains were more due to their geographic origin than due to the host species from which they originated. According to Ridom SpaServer (www.ridom.de) the most frequent *spa*-types in the world are t032, t003, t002, and t008. Asadollahi et al. (2018) have analyzed the most prevalent *spa*-types occurred human strains in particular continents and countries. Authors presented that the most prevalent *spa*-types in Europe are t008, t002, and t003, while in Poland the most frequent are *spa*-types are t003, t037, t053, t127, and t021. In contrast, in our study, the majority of human strains belonged to t084, and t9490 of *spa*-CC084.

The animal strains mostly belonged to t14395, and t010, and bovine strains were grouped. According to Ridom SpaServer (www.ridom.de) the *spa* type t010 belongs to ST5, whereas in Europe the most frequent are ST97, ST126, ST133, ST151, ST497, and ST771 (Holmes and Zadoks 2011).

Conclusions

The human and animal *S. aureus* collections were characterized by the phenotypic and molecular methods. The results obtained showed that phenotypically demonstrated resistance profiles and virulence factors were comparable to microarray's profiling.

Analysis of human and animal strains did not demonstrate any specific marker clearly differentiating them in the microarray results. However, human P-like pA+ strains were characterized by lack of *hlb*, *sak*, and enterotoxin genes in comparison to other human strains. Moreover, the bovine mastitis strains from Poland showed sensitivity to almost all antibiotics used in the project in opposite to the Slovakian ones that demonstrated a broad range of antibiotic resistance.

Our study showed that similarities between strains were more due to their geographic origin than due to the host species from which they were isolated.

ORCID

Jacek Międzobrodzki 0000-0003-4252-880X
Maja Kosecka-Strojek 0000-0001-8337-6975

Acknowledgements

The authors thank to Dr. Jarosław Król from Veterinary Department of the University of Wrocław, and Dr. Elżbieta Puacz from National Chamber of Medical Laboratory Specialists (Warszawa, Poland) for providing bacterial isolates to the presented project.

Founding

This project was partly financed by funds granted by the National Science Centre (NCN, Poland) on the basis of the decision No. UMO-2016/21/N/NZ6/00981 (to M.K.S.)

This study was partly founded by Slovak grant No. APVV-16-0171 (to V.K.).

Faculty of Biochemistry, Biophysics, and Biotechnology of the Jagiellonian University is a part of a Leading National Research Centre (KNOW), which is supported by the Ministry of Science and Higher Education in Poland.

Author contributions

M.K.S. and J.M. designed the project. J.B., A.K., K.G., L.P., V.K., V.S. and J.M. provided the strains and their data. K.L.Ł., M.K.S., J.B. performed the experiments. All authors interpreted the data. K.L.Ł., M.K.S. and J.M. composed the manuscript. All authors reviewed the manuscript.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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